

Pancreatic  $\beta$ -cell protection from inflammatory stress by the endoplasmic reticulum proteins thrombospondin 1 and mesencephalic astrocyte-derived neurotrophic factor (MANF)

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Running title: THBS1 protects  $\beta$ -cells through MANF

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#### SUPPLEMENTARY FIGURE LEGENDS

**FIGURE S1.** THBS1 knockdown increases caspase 3 cleavage in human  $\beta$ -cells. Dispersed human islet cells were transfected with negative (control) or THBS1 siRNA and exposed to thapsigargin (THA) or IL-1 $\beta$  + IFN- $\gamma$  (IL+IFN) for 24h (n=2). After treatment, cells were fixed and immunostained for insulin (green) and cleaved caspase 3 (red); nuclei were stained by Hoechst 33342 (blue).

**FIGURE S2.** THBS1 does not modulate the ATF6 pathway of the ER stress response. (A) Protein expression of ER stress markers in INS-1E cells transfected with negative (N, control) or two different THBS1 (T1 and T2) siRNAs and exposed to thapsigargin (THA) or IL-1 $\beta$  + IFN- $\gamma$  (IL+IFN) for 8h (n=3). (B) UPR luciferase reporter activity in INS-1E cells transfected with negative or THBS1 siRNA and exposed to thapsigargin or IL+IFN for 16h (n=3). (C) Protein expression of ER stress markers in INS-1E cells infected with luciferase (L, control) or THBS1 (T) adenovirus (ad) and exposed to thapsigargin or IL+IFN for 8h (n=3). (D) BiP and GRP94 mRNA expression in INS-1E cells infected with luciferase or THBS1 adenovirus and exposed to thapsigargin or IL+IFN for 16h (n=3). \*p<0.05 vs untreated cells transfected with negative siRNA or infected with luciferase-expressing adenovirus.

**FIGURE S3.** THBS1 gain- or loss-of-function does not alter oxidative stress. (A) Oxidative stress measured by DCF oxidation in INS-1E cells transfected with negative (control) or THBS1 siRNA and exposed to thapsigargin (THA) or IL-1 $\beta$  + IFN- $\gamma$  (IL+IFN) for 3h (n=3). (B) Oxidative stress measured by DCF oxidation in INS-1E cells infected with luciferase (control) or THBS1 adenovirus and exposed to thapsigargin or IL+IFN for 3h (n=3). (C) GSTm1, catalase and SOD2 mRNA expression in INS-1E cells transfected with negative or THBS1 siRNA and exposed to thapsigargin or IL+IFN for 16h (n=3). (D) GSTm1, catalase and SOD2 mRNA expression in INS-1E cells infected with luciferase or THBS1 adenovirus and exposed to thapsigargin or IL+IFN for 16h (n=3). (E) ARE reporter activity in INS-1E cells transfected with negative or THBS1 siRNA and exposed to thapsigargin or IL+IFN for 16h (n=3). \*p<0.05 vs untreated cells transfected with negative siRNA or infected with luciferase-expressing adenovirus.

**FIGURE S4.** MANF knockdown induces caspase 3 cleavage in clonal human  $\beta$ -cells. EndoC- $\beta$ H1 cells were transfected with negative (control) or MANF siRNA and exposed to thapsigargin (THA) or IL-1 $\beta$  + IFN- $\gamma$  (IL+IFN) for 24h (n=4). After treatment, cells were fixed and immunostained for insulin (green) and cleaved caspase 3 (red); nuclei were stained by Hoechst 33342 (blue).

**FIGURE S5.** MANF knockdown increases caspase 3 cleavage in human  $\beta$ -cells. Dispersed human islet cells were transfected with negative or MANF siRNA and exposed to thapsigargin (THA) or IL-1 $\beta$  + IFN- $\gamma$  (IL+IFN) for 24h (n=2). After treatment, cells were fixed and immunostained for insulin (green) and cleaved caspase 3 (red); nuclei were stained by Hoechst 33342 (blue). Panels I-P are also shown in Figure 4I.

**FIGURE S6.** Expression of Bcl-2 family proteins in THBS1 silenced or overexpressing  $\beta$ -cells. (A) DP5 and PUMA mRNA expression in INS-1E cells transfected with negative (control) or THBS1 siRNA and exposed to thapsigargin (THA) or IL-1 $\beta$  + IFN- $\gamma$  (IL+IFN) for 16h (n=3). (B) Bcl-2 and Bcl-XL protein expression in INS-1E cells transfected with negative (N) or two different THBS1 (T1 and T2) siRNAs and exposed to thapsigargin or IL+IFN for 16h (n=3). (C) Densitometric quantification of BIM protein expression in INS-1E cells transfected and treated as in panel B (n=3). (D) DP5 and PUMA mRNA expression in INS-1E cells infected with luciferase (L, control) or THBS1 adenovirus (T) and exposed to thapsigargin or IL+IFN for 16h (n=3). (E) Bcl-2 protein expression in INS-1E cells infected and treated as in panel D (n=3). \*p<0.05 vs untreated cells transfected with negative siRNA or infected with luciferase-expressing adenovirus. #p<0.05 vs thapsigargin- or cytokine-treated cells transfected with negative siRNA.

## SUPPLEMENTARY TABLES

TABLE S1: Primer sequences

Gene	Species	STD or qRT	Forward primer	Reverse primer
$\beta$ -actin	Human	STD	AAATCTGGCACCAACACCTTC	CCGATCCACACGGAGTACTT
		qRT	CTGTACGCCAACACAGTGCT	GCTCAGGAGGAGCAATGATC
$\beta$ -actin	Mouse	STD	AGAGGGAAATCGTGCCTGAC	TCTCCTCTGCATCCTGTCA
		qRT	ACGGCCAGGTCTACTATT	GTTGGCATAGAGGTCTTACG
BiP	Rat	STD	CTCAAAGAGCGCATTGACA	AATGCTATAGCCAAAGTGGCT
		qRT	CCACCAGGATGCAGACATTG	AGGGCCTCCACTTCCATAGA
Catalase	Rat	STD	CGACCAGATGAAGCAGTGG	AATCGGACGGCAATAGGAGT
		qRT	CCCTCAGAAACCCGATGTC	CACATCTGAACGAGGAGGG
DP5	Rat	STD	GCACCCTGTGACCTTCCTAA	TCACATGCACGAACACACAC
		qRT	GCCGTGGTGTACTTGGA	GATTGTGCCAGAGCTTCACA
GAPDH	Rat	STD	ATGACTCTACCCACGGCAAG	TGTGAGGGAGATGCTCAGTG
		qRT	AGTCAACGGCACAGTCAAG	TACTCAGCACCAGCATCACC
GRP94	Rat	STD	TCACAGACACGGGTGTAGGA	TCTTCTCCAAGGGCTCCTC
		qRT	AAGGTATTGTCA CGTCGAAA	GTGTTTCTCTTGGGTCAAGC
GSTM1	Rat	STD	CGCCAGAACCATGCCTAT	CTTCTCAAAGTCGGGTTGTA
		qRT	TGGGATACTGGAACGTCCG	TGTATAGTCGGGAGCGTC
MANF	Human	STD	TCGGTTGTGCTACTATATCGG	TCATCAGGAAAGCTCCAGGC
		qRT	CACAGATGATGCAGCCACCA	CAGGTCGATCTGCTTGTACATAC
PUMA	Rat	STD	TGGGTGCACTGATGGAGATA	AACCTATGCAATGGGATGGA
		qRT	AGTGCCTTCACTTTGG	CAGGAGGCTAGTGGTCAGGT
SOD2	Rat	STD	CCGTGGTGGGTGTTTAG	GGGTCTGTTGATTGTTCACTAG
		qRT	GCCCCAACACAGAGATGGAATA	TGGTGTGAGCTGCTCTGATTG
THBS1	Human, mouse	STD	CACTGATATGGATGGGTTGG	TGCAGTTGCTTGTCA
		qRT	CCGGATCAGCTGGACTCTGACT	CATGGTCAGCCTGGTGGCA

STD: primers used for conventional PCR, qRT: primers used for real time qRT-PCR

TABLE S2: Antibodies used for Western blot

Antibody	Dilution	Company	Reference	Source
THBS1	1:1000	Thermo Scientific	MA5-13385	Mouse
MANF	1:1000	Millipore	ABN306	Rabbit
Cleaved caspase 3	1:1000	Cell Signaling	9661S	Rabbit
Cleaved caspase 9	1:1000	Cell Signaling	9507S	Rabbit
Phospho-eIF2 $\alpha$ (Ser51)	1:2000	Cell Signaling	3597	Rabbit
eIF2 $\alpha$	1:2000	Cell Signaling	5324	Rabbit
ATF4	1:1000	Cell Signaling	11815	Rabbit
CHOP	1:1000	Cell Signaling	2895	Mouse
IRE1	1:1000	Cell Signaling	3294	Rabbit
XBP1s	1:1000	Santa Cruz Biotechnology	Sc-7160	Rabbit
BIM	1:1000	Cell Signaling	2819	Rabbit
ATF3	1:1000	Santa Cruz Biotechnology	Sc-188	Rabbit
GRP94	1:2000	Cell Signaling	2104	Rabbit
BiP	1:3000	Cell Signaling	3183	Rabbit
Bcl-2	1:5000	Santa Cruz Biotechnology	Sc-492	Rabbit
Bcl-XL	1:5000	Cell Signaling	2764	Rabbit
Tubulin	1:5000	Sigma	T9026	Mouse
$\beta$ -actin	1:2000	Cell Signaling	4967	Rabbit

Figure S1

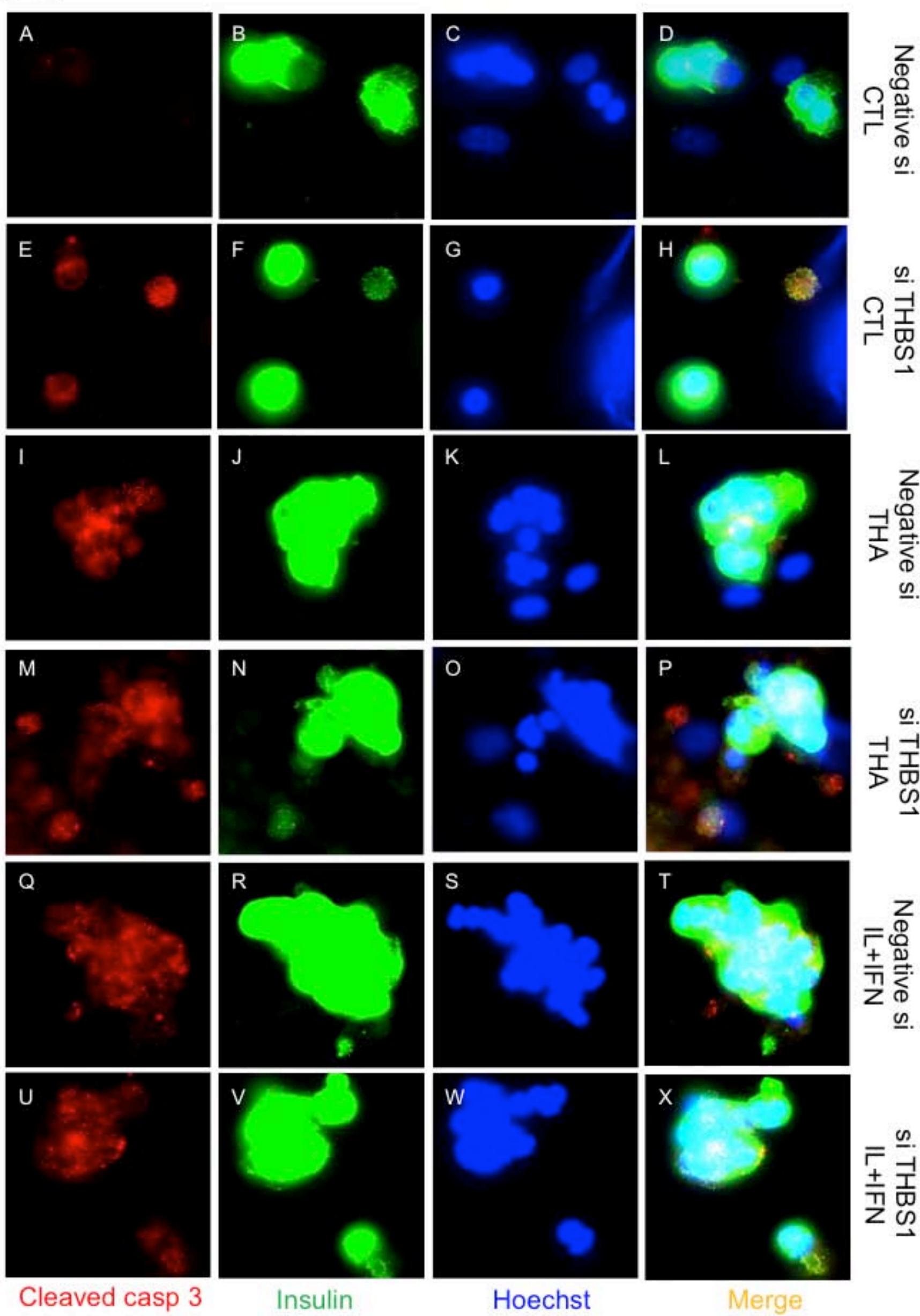


Figure S2

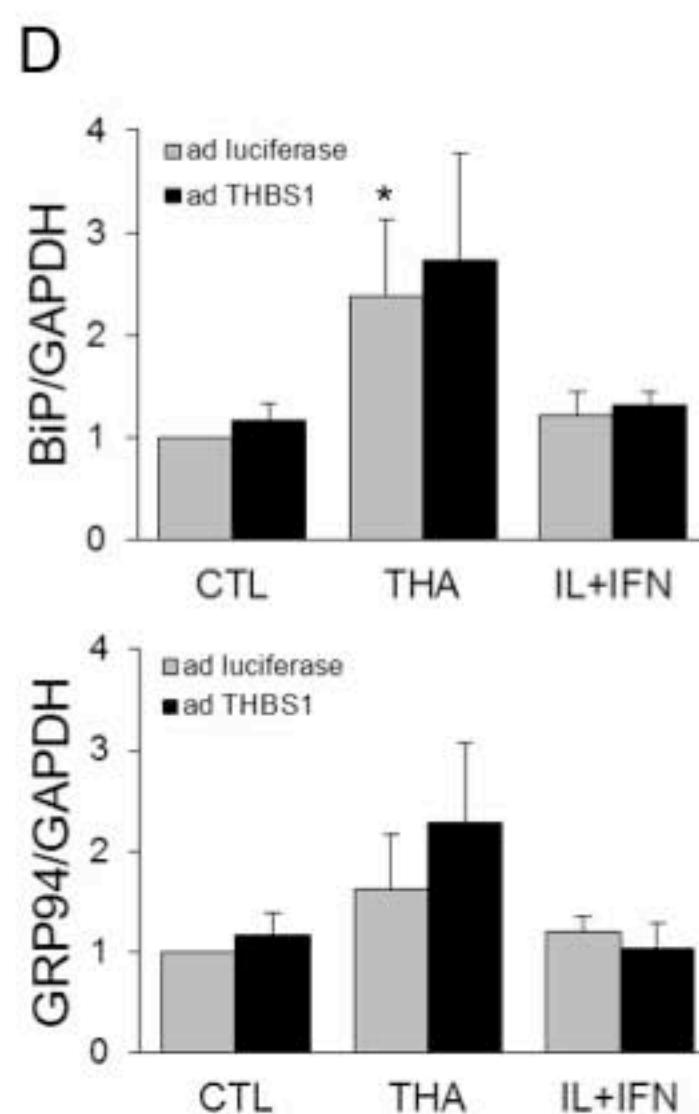
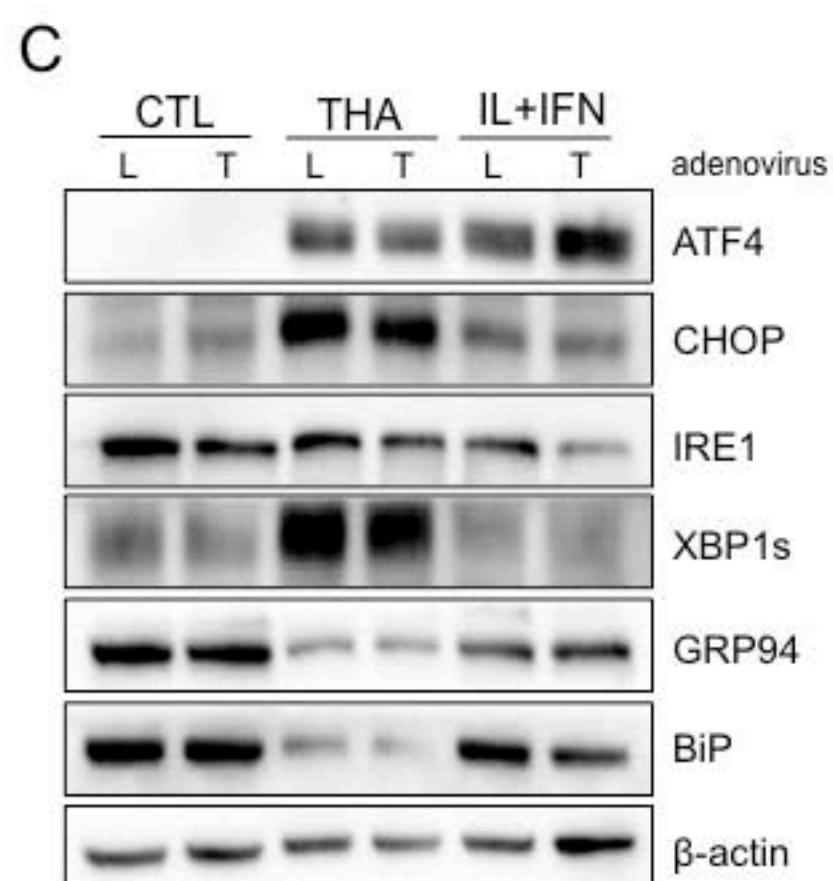
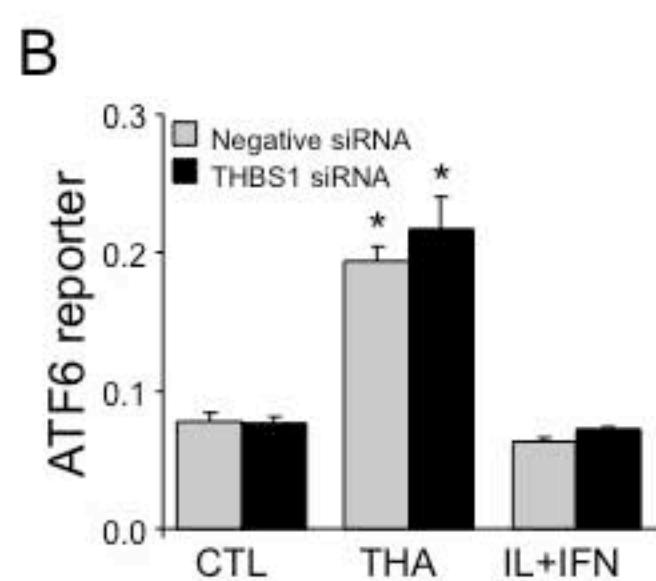
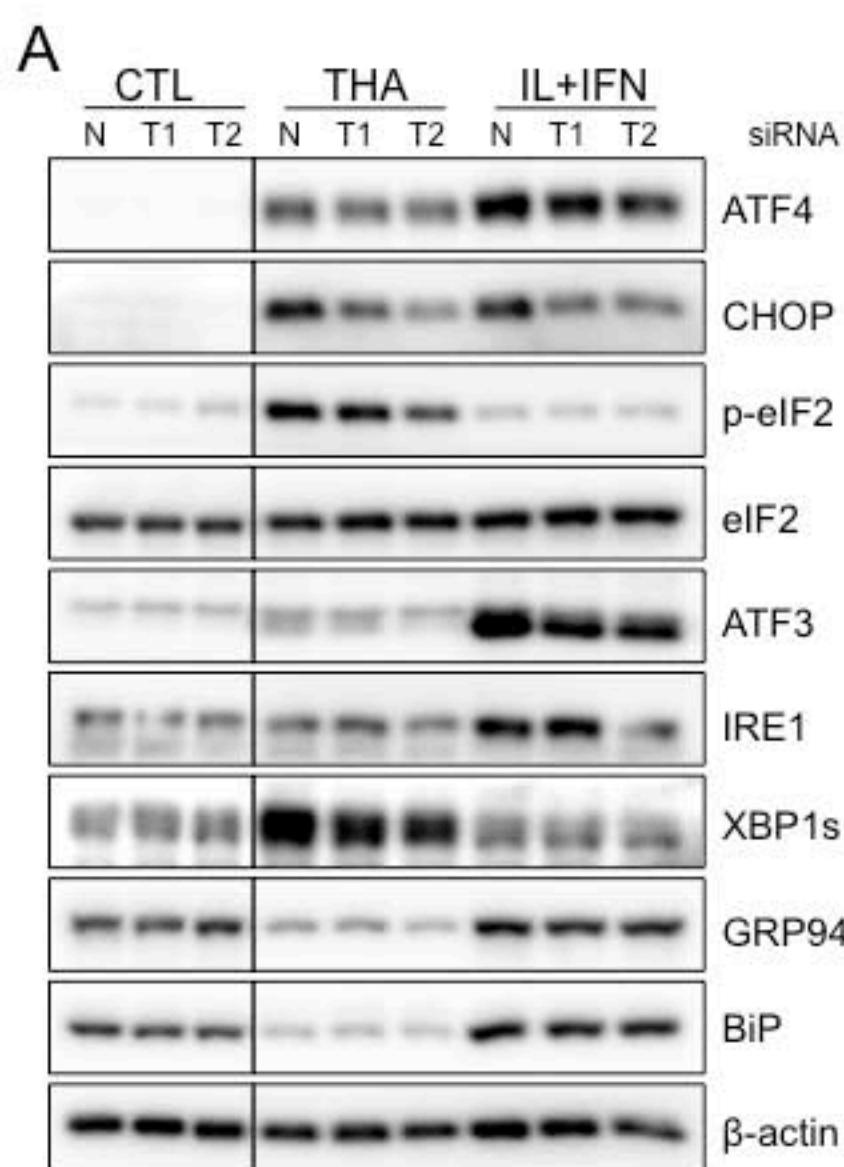


Figure S3

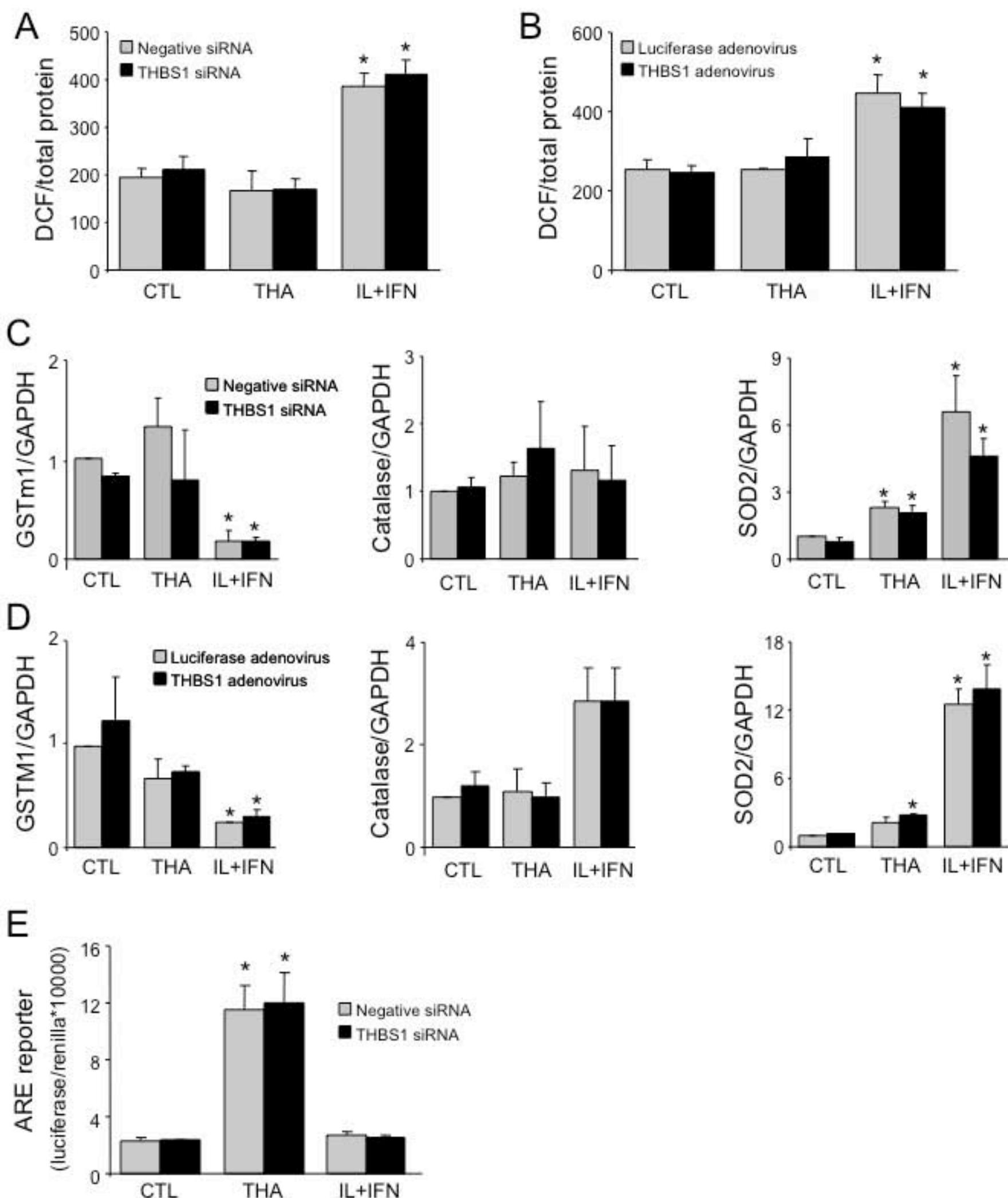


Figure S4

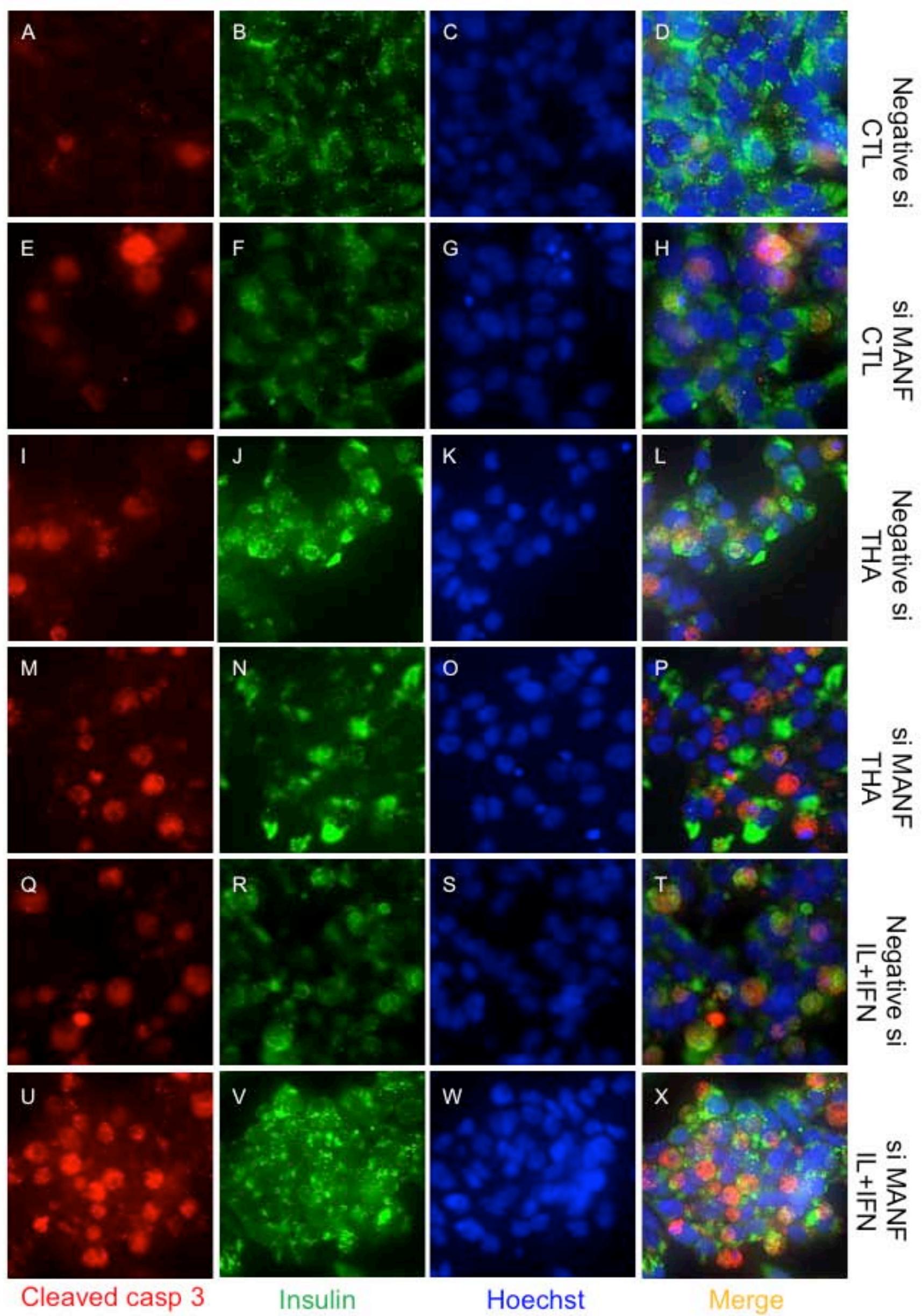


Figure S5

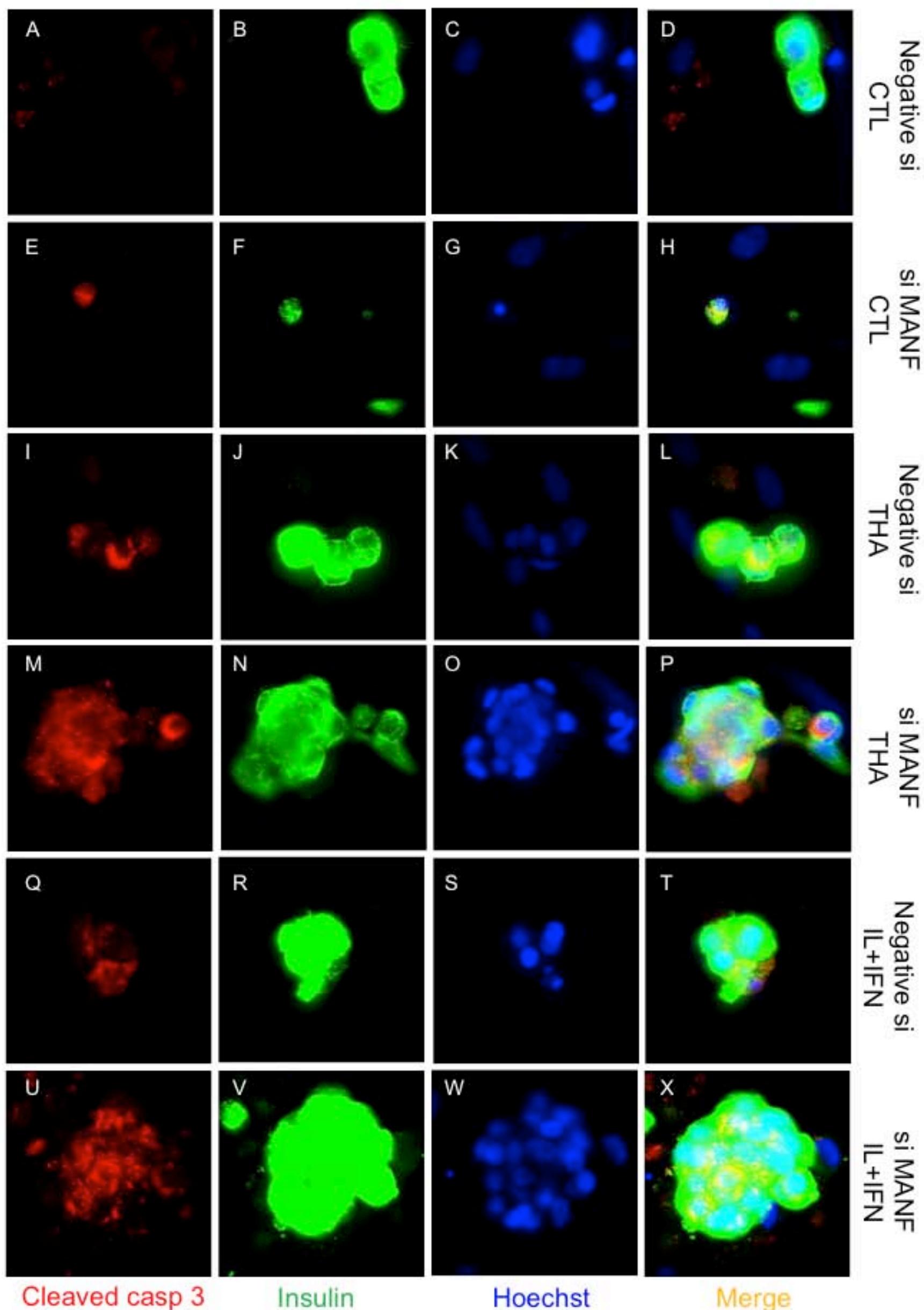


Figure S6

